The findings reported by Jones *et al.* are of importance when considering the optimal therapeutic strategy to initiate in a given patient with CTCL. Because methylation of the Fas promoter is not observed in all patients with SzS, the use of hypomethylating drugs may not be equally effective in restoring sensitivity to Fas-mediated apoptosis in all patients. Thus, not only is the existence of potent hypomethylating agents important, but their use will require a "personalized" medical approach in which these agents are employed in patients having tumors with positional methylation of the Fas CpG island.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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See related article on pg 1040

How Does Intramolecular Epitope Spreading Occur in BPAG2 (BP180)?

Takashi Hashimoto[1], Takahiro Hamada[1], Teruki Dainichi[1], Norito Ishii[1], Tadashi Karashima[1], Takekuni Nakama[1] and Shinichiro Yasumoto[1]

Several studies have suggested that autoantibodies directed against multiple epitopes occur via epitope spreading in autoimmune bullous skin diseases. However, the precise sequence of events in epitope spreading has not been elucidated for any of the epidermal autoantigens. In this issue, using a transgenic mouse model, Di Zenzo *et al.* report that intramolecular epitope spreading does occur for human BPAG2.

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In order to investigate the mechanism of epitope spreading for BPAG2 (BP180 or type XVII collagen), Di Zenzo *et al.* (2010, this issue) performed a sophisticated set of experiments using transgenic mice harboring human BPAG2. To immunize mice with human BPAG2, skin samples from transgenic mice that expressed human BPAG2 were grafted onto syngeneic mice. Sequential serum samples were then obtained from the immunized mice, and antibodies against human BPAG2 were detected by an enzyme-linked immunosorbent assay using recombinant proteins for four intracellular domains (ICDs) and three extracellular domains (ECDs) of human BPAG2. Most grafted mice

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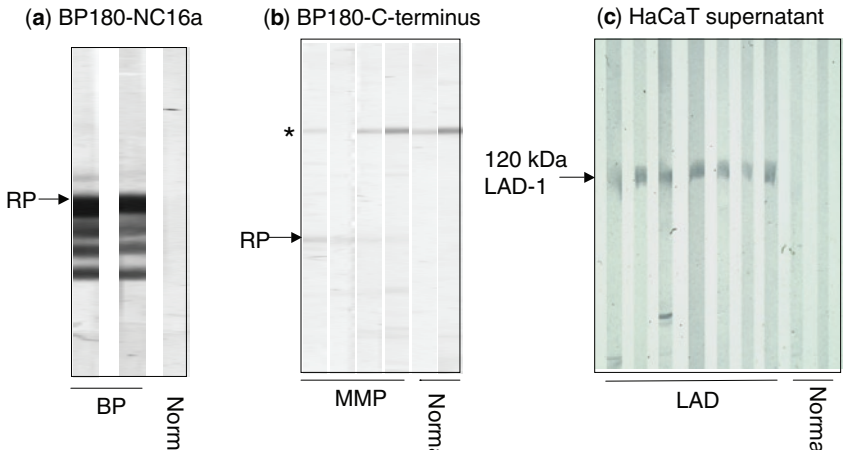

Figure 1. Representative results of immunoblot analyses for BPAG2 (BP180) using three different antigen sources. (**a**) Bacterial recombinant protein of the NC16a domain of BPAG2. IgG antibodies in bullous pemphigoid (BP) sera reacted with this recombinant protein (RP). (**b**) Bacterial recombinant protein of C-terminal domain of BPAG2. IgG antibodies in anti-BP180-type mucous membrane pemphigoid (MMP) sera reacted with this RP. The upper protein band marked with an asterisk indicates nonspecific reactivity because it is also shown by normal controls. (**c**) Concentrated supernatant sample from cultured HaCaT cells. IgA antibodies in lamina lucida type of linear IgA bullous dermatosis (LAD) sera reacted with the 120-kDa LAD-1 antigen.

initially developed anti-BPAG2 antibodies directed against ECD epitopes. Subsequently, some of the mice developed antibodies to additional ECD epitopes and to ICD epitopes. In general, the titers of antibodies against the ECD epitopes were high, whereas antibodies against the ICD epitopes were low, and they were detectable for shorter periods of time. An interesting observation was that the development of antibodies against ICD epitopes correlated with graft loss, but rejection occurred by an unknown mechanism. Thus, Di Zenzo *et al*. confirmed successfully and directly that epitope spreading does occur in this animal model of an autoimmune bullous skin disease.

Multiple epitopes occur in the autoantigens that characterize autoimmune bullous diseases

Epitope spreading has been shown to occur in several autoimmune bullous skin diseases (Chan *et al*., 1998). The multiple epitopes on desmoglein 1 (Dsg1) or Dsg3 are targets of antibodies found in sera from patients with pemphigus vulgaris, pemphigus foliaceus, and paraneoplastic pemphigus as determined by ELISA assays using domain-swapped molecules between human Dsg1 and Dsg3 (Futei *et al*., 2003). Recently, these results were confirmed in an ELISA assay using newly elaborated domain-swapped molecules of human Dsg1 and Dsg3 against the human Dsg2 backbone (Chan *et al*., in press; B Ohyama *et al*., personal communication). Previously, we demonstrated that paraneoplastic pemphigus sera had autoantibodies against multiple epitopes in human envoplakin and periplakin, two major autoantigens found in paraneoplastic pemphigus, as demonstrated by an ELISA assay, using bacterial recombinant proteins from various domains of envoplakin and periplakin (Nagata *et al*., 2001). We showed that bullous pemphigoid sera had autoantibodies against multiple epitopes in the various domains of human BPAG1 (BP230), particularly to the C-terminal globular domain, by immunoblot analysis using bacterial recombinant proteins of various domains of human BPAG1 (Hamada *et al*., 2001). We also showed that anti-basement membrane zone antibodies in the sera of patients with epidermolysis bullosa acquisita reacted with distinct epitopes in the NC1 domain, the central collagenous domain, and the NC2 domain by immunoblot analysis using bacterial recombinant proteins from selected domains of human type VII collagen and immunoelectron microscopy (Ishii *et al*., 2004).

These studies strongly suggest that intramolecular epitope spreading occurs in several autoimmune bullous skin diseases. Although the previous studies detected autoantibodies against multiple epitopes, no study detailed the sequential development of autoantibodies to different epitopes over time. Di Zenzo *et al*. (2010) have now shown that epitope spreading actually takes place. They found that mice immunized against human BPAG2 occasionally developed autoantibodies against some epitopes present in the ICD of BPAG2, although such autoantibodies appeared late and were less persistent. Interestingly, we found that pemphigus patients sometimes have autoantibodies that react against the ICD of Dsg1 and Dsg3 (Ohata *et al*., 2001). Thus, the study by Di Zenzo *et al*. also confirms that antibodies against the ICD of transmembranous antigens can occur in autoimmune bullous skin diseases, probably also by epitope-spreading mechanisms. The mechanism by which such antibodies develop remains unknown.

BPAG2 is the most suitable autoantigen for studying mechanisms of epitope spreading

Autoantibodies to distinct epitopes within BPAG2 develop in a variety of autoimmune subepidermal bullous skin diseases. First, anti-basement membrane zone autoantibodies in both bullous pemphigoid and herpes gestationis were reported to preferentially react with the NC16a domain of BPAG2 (Matsumura *et al*., 1996). In addition, IgG and IgA antibodies in anti-BP180 type mucous membrane pemphigoid have been shown to react with the C-terminal domain of BPAG2 (Nie and Hashimoto, 1999). Furthermore, we showed that IgA antibodies in lamina lucida–type linear IgA bullous dermatosis reacted with epitope(s) within the fifteenth collagenous domain of BPAG2, which is hidden in the intact 180-kDa BPAG2 molecule (Nie *et al*., 2000). For these reasons, BPAG2 is considered the most suitable antigen with which to elucidate mechanisms of epitope spreading in autoimmune bullous skin diseases. Figure 1 shows representative immunoblot analyses for three antigen sources used routinely in our laboratory.

Clinical Implications

- Epitope spreading is the sequential development of new antibodies against seemingly less accessible regions of target proteins in autoimmunity.
- The identification of mechanisms of epitope spreading in the immunobullous diseases may lead to novel therapies that limit the process of spreading.
- Because of accessibility, the analysis of epitope spreading in skin disease may provide insight into pathogenic mechanisms in systemic autoimmune diseases and transplantation immunity.

Perspectives

Although Di Zenzo *et al*. (2010) demonstrated convincingly that intramolecular epitope spreading occurs in BPAG2, many questions remain. The first is why patients with bullous pemphigoid preferentially develop IgG autoantibodies to epitopes on the NC16a domain of BPAG2. Second, why do autoantibodies in bullous pemphigoid react with epitopes in the NC16a domain of BPAG2, whereas autoantibodies in anti-BP180–type mucous membrane pemphigoid react with epitopes in the C-terminal domain? More important, how do the antibodies directed against these distinct domains of BPAG2 result in different clinical features (i.e., large, tense skin blisters in bullous pemphigoid and predominant erosive mucosal lesions in anti-BP180-type mucous membrane pemphigoid)? Why do IgA antibodies in lamina lucida–type linear IgA bullous dermatosis react with specific epitopes in 120- and 97-kDa linear IgA bullous dermatosis (LAD)-1 antigens produced from 180-kDa intact BPAG2 by proteolytic processing (Nie *et al*., 2000)? Future studies should unravel the mechanisms by which the hidden epitope in intact 180-kDa molecule (intact BPAG2) is exposed in linear IgA bullous dermatosis to autoantibodies against the 120- and 97-kDa LAD-1 antigens.

Finally, and perhaps most important, we do not know yet why the development of antibodies against ICD epitopes in human BPAG2 correlated with skin-graft loss. The relevance of this phenomenon to autoimmune bullous diseases remains to be determined.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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See related article on pg 1126

More or Less: Copy Number Alterations in Mycosis Fungoides

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Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma (CTCL), a heterogeneous group of non-Hodgkin's lymphomas of skin-homing T cells. MF may vary from limited patchy skin disease to extensive cutaneous plaque and tumor involvement to extracutaneous compartments of blood, lymph nodes, and viscera. Advances in genomic technologies have enabled the increasing characterization of genetic alterations in this malignancy; using this technology, investigators hope to understand MF's variable behavior and pathogenesis. In this issue, Salgado *et al.* identify regions of genomic DNA alterations from 41 MF samples and report associations with prognosis.

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In recognition that cancer is fundamentally dependent on genetic alterations (Vogelstein and Kinzler, 2004), the number of genomic

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